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High immunogenicity of red blood cell antigens restricted to the population of African descent in a cohort of sickle cell disease patients

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Statement of equal author's contribution:

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Abstract

BACKGROUND

Sickle cell disease (SCD) patients undergo multiple red blood cell (RBC) transfusions and are regularly exposed to low-prevalence (LP) antigens specific to individuals of African descent. This study evaluated the prevalence of antibodies (Abs) against LP antigens in SCD patients, and the need to identify these Abs in everyday practice.

STUDY DESIGN AND METHODS

Plasma from 211 SCD patients was tested with RBCs expressing the following LP antigens: RH10 (V), RH20 (VS), RH23 (D^W), RH30 (Go^a), KEL6 (Js^a) and MNS6 (He).

RESULTS

Nine anti-LP Abs were found in eight patients (3.8%): five anti-RH23, two anti-RH30, two anti-MNS6. The exposure risk, calculated for each LP antigen, was below 3% per packed RBC, for all antigens tested. Thus, in this cohort of transfused SCD patients, the prevalence of anti-LP Abs was similar to that of Abs against Ag of the FY, JK and MNS blood group systems. These findings also reveal the occurrence of anti-RH23 in SCD patients. No anti-RH20 or anti-KEL6 were found, despite the high frequency of mismatch situations.

CONCLUSION

These results highlight the immunogenicity of these LP antigens, and the evanescence of Abs against LP antigens. They also highlight the importance of appropriate pre-transfusion testing for patients frequently transfused, who are likely to be exposed to multiple types of blood group antigens.

Key words: Transfusion, low-prevalence antigens, sickle cell disease, immunogenicity, alloimmunization

Introduction

Sickle cell disease (SCD) is the most frequent genetic disease in France. Many affected patients require regular transfusions of packed red blood cells (pRBCs) to limit complications and extend patient life-span.¹ Alloimmunization is one of the main complications of transfusion. It can lead to poor transfusion efficacy, a lack of compatible donors, or even life-threatening conditions, such as delayed hemolytic transfusion reaction (DHTR).² To reduce alloimmunization, RH- (D, C, E, c, e) and KEL1-matched pRBCs are selected for all SCD patients in France, and systematic pre-transfusion cross-match tests are performed.

There is marked red blood cell (RBC) antigen polymorphism between blood donors in France (mostly of Caucasian descent) and SCD patients (mostly of African descent). Some RBC antigens are more prevalent in donors than in patients: C, Fy^a, Jk^b, S. The corresponding antibodies are readily detectable in pre-transfusion screening tests with standard test RBCs. Other RBC antigens are almost exclusive to Afro-Caribbean populations, due to specific polymorphisms found in several blood group systems: RH (RH10 or V, RH20 or VS, RH23 or D^W, RH30 or Go^a...), MNS (MNS6 or He), KEL (KEL6 or Js^a).³ Despite a prevalence of over 1% in individuals of African descent, these antigens are said to be of low-prevalence (LP), based on their rarity in the general donor population (mostly of Caucasian descent). These LP antigens can induce alloimmunization and the production of specific Abs. The risk of alloimmunization is higher in SCD patients than in other transfusion recipients, due to their specific inflammatory status,⁴ the frequency of transfusion, and the need to match donor and recipient phenotypes, resulting in a higher probability of SCD patients being exposed to pRBCs from donors of African descent.^{5,6}

Alloimmunization to LP antigens can be detected only in extensive studies at specialized reference centers, because these rare antigens are not included in the standard RBC panels used for pre-transfusion screening tests. In practice, these antibodies are sought only in specific situations: DHTR, polyimmunization or positive cross-match test. The occurrence and characteristics of anti-LP antibodies in the general population of SCD patients receiving transfusions therefore remain unknown. In France, cross-match tests are performed before each blood transfusion in SCD patients, to facilitate the detection of Abs against LP antigens.

It is currently impossible to predict individual alloimmunization risk. Antigen immunogenicity^{7,8} is, therefore, an important factor guiding decisions in transfusion medicine, together with the possible clinical consequences of alloimmunization. Antigen immunogenicity indicates the likelihood of an antigen inducing alloantibody formation. In previous studies, the immunogenicity of the KEL1 antigen was used to standardize immunogenicity calculations⁹⁻¹¹ in situations in which KEL1-matching was not systematic.

The principal aim of this study was to determine the prevalence of antibodies directed against LP antigens specific to the Afro-Caribbean population, in SCD patients receiving blood transfusions. Our secondary aim was to estimate the immunogenicity of these antigens, by calculating antigen exposure rates.

Materials and Methods

Study design

SCD patients with a history of one or more RBC transfusions were included. Each patient underwent molecular testing to predict their RH10, RH20 and KEL6 phenotypes. Each sample from each patient was tested for Abs against the LP antigens RH23, RH30 and MNS6, as well

as for Abs against RH10, RH20 and KEL6 for patients with a respective negative phenotype (Fig. 1).

Patients and samples

All SCD patients with a history of transfusions referred to the *Etablissement Français du Sang (EFS) Ile-de-France* (Créteil) for routine antibody screening by the Henri Mondor University Hospital SCD Center (Créteil, France) and the pediatric center of the Créteil Intercommunal Hospital (CHIC, Créteil, France) between November 2013 and March 2014 were eligible for inclusion in the study.

This study was approved by CPP IDF IX/IV (the competent medical ethics committee) under agreement number 10–040.

The sex and age of the patients, together with their transfusion history and previous antibody screening test results, were recorded from the *EFS Ile-de-France* information system, and all data were anonymized to protect the patients' privacy and confidentiality. The patients had SS, SC, or S- β -thalassemia forms of the disease.

The remainder of the EDTA-anticoagulated blood samples from these patients left over after routine testing was collected. Routine antibody screening and identification was performed in accordance with French recommendations,¹² on each sample, on the day of inclusion into the study.

Choice of Afro-Caribbean-specific antigens

Six Afro-Caribbean-specific RBC antigens were selected for this study on the basis of their high prevalence in Afro-Caribbean populations or the availability of test RBCs carrying these

antigens at the time of the study (Table 1): RH10 (V), RH20 (VS), KEL6 (Js^a), RH23 (D^w), RH30 (Go^a), and MNS6 (He). Table 1 summarizes the main characteristics of the antigens selected, and the molecular backgrounds in which they are expressed.

Molecular analysis

The expression of the three most frequent Afro-Caribbean LP antigens (KEL6, RH10 and RH20) was predicted by molecular analysis for each patient included in the study. We extracted gDNA from leukocytes (Magnapure, Roche, France). Real-time polymerase chain reaction (PCR) assays (5' nuclease assay, Thermo Fisher, USA) were then performed, as previously described.¹⁸ The c.712A>G, c.733C>G and c.1006G>T polymorphisms of the *RHCE* gene (in exons 5 and 7), and c.1790T>C of the *KEL* gene (exon 17) were detected. We deduced the expression of the RH10 and RH20 antigens from these results (see supplemental data).

Samples from 1000 FY:-1,-2 mainland French blood donors were screened by molecular analysis to deduce their RH20 and KEL6 antigen expression. For RH20, only the c.733C>G *RHCE* polymorphism was explored on such a large scale. In this way, we were able to estimate the prevalence of the RH20 and KEL6 antigens in mainland French donors of African origin.

Serological analysis

We selected a panel of test RBCs expressing the Afro-Caribbean-specific antigens from the collection of the National Immunohematology Reference Laboratory (*Centre National de Référence pour les Groupes Sanguins*). The test RBCs expressed the following antigens: RH10 and RH20 for test-RBC no. 1, KEL6 and RH23 for no. 2, RH30 for no. 3, MNS6 and RH23 for no. 4.

All samples were screened with this in-house panel using an IgG indirect antiglobulin test (IAT) with untreated and papain-treated RBCs on a gel matrix (Bio-Rad, USA). If molecular analysis predicted the expression of RH10 and RH20 for a patient, the samples from this patient were not screened with test RBC no. 1. Antibody specificities were confirmed with at least two more informative test RBCs expressing the target antigen, and with several test RBCs negative for the antigen.

Antibody adsorption test was performed in accordance with the standard procedures when autoantibodies made it difficult to interpret the antibody screening results.

Immunogenicity and antigen exposure

In this study, we calculated an antigen exposure risk for each pRBC unit received, with a method derived from the equation established by Giblett in 1961.⁹ This antigen exposure risk was calculated with no reference to anti-KEL1 alloimmunization, because SCD patients are not exposed to KEL1 antigen in France (systematic KEL1 match) and receive variable numbers of pRBCs, resulting in considerable variation in the frequency of exposure to LP antigens.

Antigen immunogenicity was then compared by matching the calculated antigen exposure rate and alloimmunization rate for each antigen within our cohort. A lower rate of antigen exposure associated with the same number of alloimmunized patients indicated higher antigen immunogenicity.

Antigen prevalence differs between ethnic backgrounds, and exposure rates are therefore influenced by the frequency with which SCD patients receive pRBCs from donors of different ethnic backgrounds. The risk of exposure to Afro-Caribbean LP antigens is considered to be

zero for pRBCs from Caucasian donors, and the frequency with which SCD patients receive Afro-Caribbean pRBCs ("intra-ethnic transfusions") is currently estimated at 11.5% in mainland France.¹⁹

The exposure risk (R) for an antigen per pRBC unit received was calculated as follows: $R = P_{\text{donor}} * F * (1 - P_{\text{patient}})$, where P_{donor} and P_{patient} are the prevalences of the antigen in the donor and patient populations, respectively ($P_{\text{donor}} = P_{\text{patient}}$ if the donor and patient have the same ethnic background) and F is the frequency with which SCD patients receive pRBCs from Afro-Caribbean donors.

Results

Patients and samples

We included 211 SCD patients in this study. During the collection period, two patients were excluded because they had previously received an allograft of hematopoietic stem cells (HSCs), and two patients were excluded because their DNA (extracted from a single sample with no follow-up sample) was of too low quality for molecular analysis.

Patients had received between 2 and 872 pRBCs (mean: 144) before their enrolment in the study.

For each patient, 1 to 12 successive samples (mean: 1.84 samples per patient) were included in the serological analysis during the collection period. Overall, 389 samples were screened during this study.

Antibody history and antibody screening at inclusion

The patients' antibody history and antibody screening at inclusion are detailed in Table 2. The antibodies against the main RH antigens (D, C, E, c, e) in our cohort result from: (i) transfusion with pRBCs in other countries in which antigen-matching protocols are not performed for these antigens, (ii) previously undetected partial RH antigens, (iii) pregnancy, or (iv) possible autoantibodies that mimic alloantibodies (so-called "mimicking alloantibodies").

Before enrolment in the study, four patients had already tested positive for antibodies against Afro-Caribbean-specific LP antigens: two patients had presented anti-RH20 Abs, one had presented anti-KEL6 + anti-RH20 Abs, and one had presented anti-RH10 + anti-RH20 + anti-RH23 Abs.

At the time of enrolment in the study, 35 patients (16.6%) had positive antibody screening tests (Table 2). Successive plasma samples were tested for some patients, and additional antibodies became detectable after enrolment in three patients. Two of these patients had positive screening tests at inclusion, with anti-RH2 in one patient and autoantibodies in the other subsequently becoming detectable. For the third patient, anti-KEL3 antibodies were detected after enrolment. In all three cases, the antibodies detected had already been detected in the past. Adsorption techniques were required for 17 samples (7 patients) presenting autoantibodies.

Molecular testing

The KEL:–6 phenotype was deduced by molecular analysis for 167 SCD patients (79.1 %). The RH:–20 phenotype was deduced for 118 patients (55.9 %), and the RH:–10 phenotype was deduced for 133 patients (63.0%). Among the 1000 FY:–1,–2 donors screened, the KEL:6 phenotype was deduced for 172 donors (17.2%), and the RH:20 phenotype was deduced for 458 donors (45.8%). These results are consistent with previous findings.³

Antibodies against LP antigens specific to the Afro-Caribbean population

KEL:6 patients were not tested for anti-KEL6 Abs, and RH:20 and RH:10 patients were not tested for anti-RH20 and anti-RH10 Abs, respectively. All patients were tested for anti-MNS6, anti-RH30 and anti-RH23 Abs.

Antibodies against Afro-Caribbean LP antigens were detected in eight patients (3.8%): anti-MNS6 + anti-RH23 Abs in one patient, anti-MNS6 Abs alone in one patient, anti-RH30 Abs in two patients, and anti-RH23 Abs alone in four patients. Anti-RH23 Abs in three patients and anti-RH30 Abs in one patient were detectable only with papain-treated test-RBCs. No anti-KEL6, anti-RH10 or anti-RH20 Abs were detected. One of the patients with anti-RH23 Abs had previously tested positive for antibodies against RH20 and KEL6, but these antibodies were no longer detectable at the time of the study.

The characteristics of the patients with antibodies against Afro-Caribbean LP antigens detected in this study (P1 to P8), or documented in the patient's medical history (P7 and P9 to P11) are reported in Table 3. No DHTR occurred during the study or during follow-up for patients undergoing further transfusions. Patients P4 and P9 had a history of DHTR.

Risk of antigen exposure

The calculated rate of antigen exposure is shown in Table 4. The prevalences of the RH10, RH20 and KEL6 antigens deduced by molecular analysis in our SCD or donor populations were used to calculate the risk of antigen exposure. For antigens RH23, RH30 and MNS6 we used antigen prevalence data from other studies (see Table 1).

For the purposes of comparison, we also estimated the risk of exposure to the FY1 (Fy^a) antigen for our cohort. This calculated risk takes into account exposure to pRBCs from both donors of Caucasian descent and donors of African descent. These donor populations have different FY1 antigen prevalence, this antigen having a much higher prevalence in individuals of Caucasian descent. We considered donors of African descent to have levels of FY1 expression similar to that in our SCD patients, most of whom originated from West and Central Africa (5.2%). The vast majority of donors in mainland France are of Caucasian descent, and the collection of ethnicity-related data is prohibited in France. It was, therefore considered reasonable to assume that the pRBCs received by SCD patients during transfusion were from a donor of African descent in 11.5% of cases ¹⁹ and from a Caucasian donor in 88.5% of cases. The calculated risk of exposure to the FY1 antigen in the SCD patients of our cohort is therefore 53.6% per pRBC unit received, consistent with previous observations. ¹⁹

Similarly, the risk of exposure to the MNS3 (S) antigen and JK2 (Jk^b) antigens was 38.6% and 40.1% per pRBC unit received, respectively.

A comparison between the rate of exposure to antigens and the number of alloimmunizations in our cohort showed the Afro-Caribbean LP antigens to be highly immunogenic (Table 5). Indeed, the rates of exposure to FY1, JK2 and MNS3 were at least 10 times those for any of the Afro-Caribbean-specific LP antigens, for similar numbers of patients displaying alloimmunization.

Discussion

Many SCD patients require regular transfusions of pRBCs to limit complications. Improvements in the management of blood transfusions in these patients through the prevention

of alloimmunization (selection of RH- (D, C, E, c, e) and KEL1-matched pRBCs for all SCD patients and systematic pre-transfusion cross-match tests) have helped to increase the life expectancy of SCD patients. However these developments raise new issues. Intra-ethnic transfusion rates are increased by antigen-matching protocols for FY, JK, MNS antigens, particularly for immunized patients, and SCD patients are increasingly being exposed to Afro-Caribbean-specific antigens, which are absent in donors of Caucasian descent and not detected during routine antibody screening, unlike LP antigens which are common in French persons of Caucasian descent and can be detected in pre-transfusion screening tests with standard test-RBCs.¹² We show here that 3.8% of SCD patients have detectable antibodies against Afro-Caribbean-specific LP antigens. These findings highlight the need to maintain cross-matching protocols for SCD patients, as long as test RBCs do not express these antigens, although this precaution is unlikely to be sufficient on its own.

Surprisingly, none of the antibodies found in this study were directed against KEL6, RH10 or RH20 antigens, despite the high prevalence of these antigens in individuals of African descent, resulting in high rates of exposure. The exposure rates calculated for RH10 and RH20 antigens were 2.68% and 2.94%, respectively, and that for KEL6 was 1.56% per pRBC unit received.

Moreover, no anti-RH10, anti-RH20 or anti-KEL6 antibodies were detected during the study in the four patients with a history of anti-KEL6, -RH10 and/or -RH20 alloimmunization. These findings suggest that antibodies against KEL6, RH10 and RH20 antigens are highly evanescent. They can be detected only over short periods, and cross-match tests may yield negative results shortly after alloimmunization. Most of the plasma samples studied here were collected from patients enrolled in chronic transfusion programs, at least one month after the previous transfusion episode, so evanescent antibodies may not have been detected.

Anti-RH23, anti-RH30 and anti-MNS6 antibodies were detected in this study. Even though all patients included underwent multiple transfusions, we cannot rule out the natural occurrence of these Ab. In any case, Ab detected in SCD patients should always be taken into account for future pRBC transfusions. The rate of anti-RH23 alloimmunization was particularly high (2.4%), given the rarity of the RH23 antigen within Afro-Caribbean populations.^{16,17} Anti-RH30 antibodies were detected consistently over the course of three months (six successive samples), with no decrease in reactivity, in one of our patients.

The calculated exposure rates for the RH23, RH30 and MNS6 antigens were all below 1% per pRBC unit received. This rate of exposure is much lower than that for the FY1 antigen (53.6% per pRBC unit received) calculated for our cohort. Despite this high rate of exposure to FY1 antigens, only five of the patients had currently detectable antibodies against FYI or a history of such antibodies. Conversely, the number of alloimmunizations directed against RH23, RH30 or MNS6 antigens detected was unexpectedly high. This shows that RH23, RH30 and MNS6 antigens are highly immunogenic.

The high immunogenicity of the RH23 and RH30 antigens, and the slower evanescence of antibodies directed against these antigens may account for the higher rate of detection of these antibodies in our study. Additional evidence for the high immunogenicity of the RH23, RH30 and MNS6 antigens is provided by the occurrence of alloimmunization in patients who had undergone only a few transfusions (< 10 pRBC units for P2 and P7, and < 30 pRBC units for P4, P9 and P10).

Antibodies against Afro-Caribbean LP antigens have been detected in previous studies, but this is the first study to have determined the prevalence of anti-RH10, anti-RH20, anti-RH23, anti-RH30, anti-KEL6 and anti-MNS6 antibodies at a given time within a cohort of polytransfused SCD patients. Some previous studies did not specify whether there had been any systematic screening for these antibodies.²⁰⁻²² The transfusion protocols in some studies were different from that used here (no matching for RH2, RH3, RH4, RH5 and KEL1 antigens,²⁰ or intra-ethnic transfusions²³ only), whereas other studies were limited to specific populations (pediatric²² or non-SCD¹⁰ patients). The findings of these studies cannot, therefore, be directly transposed onto current transfusion practices for SCD patients in our country at our hospital. In these studies, antibody prevalences in SCD patients ranged from 0.3% to 5.6% for anti-KEL6 Abs, and from 1.7% to 4.4% for anti-RH30 Abs. Not all studies distinguished between anti-RH10 and anti-RH20 Abs, and the reported prevalence of these antibodies ranged from 0.4% to 1.1%.

The results of these previous studies are consistent with our findings of low immunization rates or evanescence for anti-RH10 and anti-RH20 Abs and of high immunogenicity for RH30. The difference in anti-KEL6 immunization rates may be due to antibody screening being performed sooner after transfusion in previous studies, and due to the transfusion of ethnically matched RBCs, resulting in higher rates of exposure, in the study by Chou and colleagues.²³

The evanescence of antibodies^{7,24} and immunogenicity of antigens¹⁰ have already been studied for some Afro-Caribbean-specific antigen-antibody combinations. Antibody evanescence appears to be variable, but only a few patients with Afro-Caribbean-specific antibodies were available in the studies. In a non-SCD population,¹⁰ the immunogenicity of the KEL6, RH10 and RH30 antigens was found to be relatively high, similar to that of RH3, and almost three

times higher than that of FY1. This immunogenicity was also higher than that of the minor antigens JK2 and MNS3.

In addition to antigen immunogenicity, it is also important to consider the potential consequences of alloantibody formation. SCD patients constitute a very specific population, due to their inflammatory status and their need for multiple transfusions. They are prone to alloimmunization, and the clinical consequences can be disastrous, leading to life-threatening delayed haemolytic transfusion reactions (DHTRs). Most cases of DHTR occur in alloimmunized patients,²⁵ but it is often difficult to implicate one specific antibody. Several cases of DHTR have been imputed to anti-RH30²⁶ or anti-KEL6^{27,28} antibodies, and others have been observed following polyimmunization to several LP antigens, including RH10 and KEL6,^{29,30} RH23 and RH30.^{31,32} A case of hemolytic disease of the newborn has also been attributed to anti-KEL6 alloimmunization.³³ The greatest caution is therefore required when antibodies against LP antigens are detected in an SCD patient. Systematic testing for the presence of these antibodies in cases of DHTR should help to determine their clinical significance.

We did not include the LP antigen RH32 in this study because of the high prevalence of partial RH2 antigens in SCD patients,¹⁸ resulting in little or no RH:2 pRBCs being used for transfusions in French SCD patients in recent years. Furthermore, it was not possible to trace RH:2 pRBCs back to Caucasian or Afro-Caribbean donors, due to the frequency of the RH:2 phenotype in all ethnicities. Other Afro-Caribbean LP antigens could have been considered (e.g. RH42, RH43, RH48, RH49, RH54) but were not included in this study because of the limited availability of test RBCs carrying these antigens. Furthermore, alloimmunization to these

antigens is probably extremely rare, and no severe clinical consequences have been described for most of them.

In our country, SCD patients receive pRBCs strictly matched for the main antigens of the RH system (D, C, E, c, e) and the KEL1 antigen, and antigen-negative protocols are used for patients with partial D or C antigens. Extended antigen matching for FY, JK, MNS blood groups^{21,34,35} and intra-ethnic transfusions^{6,23,36} have been shown to reduce overall alloimmunization in SCD patients, but blood donor varies varies, depending on the country and such "high-level" strategies are difficult to implement in most settings.³⁷⁻³⁹

The risk of alloimmunization for the antigens of the FY, JK and MNS blood groups is, therefore, the main concern when managing blood transfusions in SCD patients in mainland France. However, as antigen-matching protocols for FY, JK, MNS are always recommended for immunized patients, the proportion of donors of African descent recruited in mainland France is increasing. Immunized patients are considered "high responders",⁴⁰ and immunization against Afro-Caribbean LP antigens is likely to become an increasingly important issue as the proportion of intra-ethnic transfusions increases.⁴¹ In the most extreme case, if all SCD patients received pRBCs from donors of African descent, the rate of exposure to LP antigens would increase to as much as 24.6% per pRBC unit received for the most prevalent Ag (RH20), whereas FY1 exposure rates would be only 5% per pRBC unit received. Routine antibody screening would be inefficient, and the frequency of undetectable antibodies against LP antigens would increase. The high immunogenicity of some of these antigens and the evanescence of Abs, as shown here, might also create new difficulties in the management of blood transfusions for patients, which we need to anticipate.

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Authorship contributions

FP, TP and AF designed the study. AF and DG performed the molecular and serologic analyses. CT supervised the technical analyses and made substantial contributions to conception and design. AH and BC co-ordinated the collection of samples and data. AF, FP, CT, TP analyzed and interpreted the results. AF and FP wrote the manuscript, which all authors critically reviewed.

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TABLES

Table 1:

Ag	Common name	Gene(s)	Polymorphism(s) necessary for the Ag's expression, or allele	Ag prevalences:	
				in Afro-Caribbeans	in Caucasians or other populations
RH10	V	<i>RHCE</i>	c.733G and c.1006G	30%	Caucasians: 1%
RH20	VS	<i>RHCE</i>	c.733G	26 to 40%	Other populations: <0.01%
RH23	D ^W (Weil)	<i>RHD</i>	<i>RHD</i> alleles with all or part of <i>RHCE</i> exon 5	1 - 3,6%	All populations: <0.01%
RH30	Go ^a	<i>RHD</i>	allele <i>RHD*04.01</i> (or <i>DIVa</i>)	2%	Caucasians: <0.01%
KEL6	Js ^a	<i>KEL</i>	c.1790C	20%	Caucasians: <0.01%
MNS6	He	<i>GYPB</i> or hybrid alleles	alleles <i>GYPB*06.01</i> , <i>GYPB*06.02</i> , <i>GYPB*03N.02</i> , <i>GYPB*03N.04</i>	3 to 7%	Caucasians: 0%
	(Henshaw)	within the <i>GYP</i> gene family	or hybrid alleles <i>GYP*101.04</i> or <i>GYP*505</i>		

Table 1: Main characteristics of the selected antigens specific to the Afro-Caribbean population, according to Reid and colleagues,³ except for the polymorphisms necessary for RH23 Ag expression and its prevalence in Afro-Caribbeans.¹³⁻¹⁷(Ag : antigen)

Table 2:

Ab specificity	Ab history <i>n</i> (%)	Ab screening at inclusion <i>n</i> (%)
No Ab	98 (46.4%)	181 (85.8%)
Abs of low clinical significance*	60 (28.4%)	21 (10.0%)
RH and KEL1 †	28 (13.2%)	7 (3.3%)
Other important specificities (JK, FY, MNS, DO) †	total: 12 (5.7%)	total: 0
- anti-FY1	2 / 12	
- anti-JK2	3 / 12	
- anti-MNS3	3 / 12	
- anti-MNS3 + anti-JK2	1 / 12	
- anti-FY3 + anti-JK2	1 / 12	
- anti-FY3 + anti-MNS3 + anti-JK2 + anti-DO1	1 / 12	
- anti-DO1	1 / 12	
RH and KEL1 + other important specificities (JK, FY, MNS, DO) †	total: 13 (6.2%)	total: 2
- anti-JK1	0	1 / 2
- anti-FY1	1 / 13	
- anti-FY1 + anti-FY5	1 / 13	
- anti-FY3	1 / 13	
- anti-FY1 + anti-JK2	1 / 13	
- anti-FY1 + anti-MNS3	1 / 13	
- anti-JK1 + anti-MNS4	2 / 13	
- anti-JK2	1 / 13	
- anti-JK2 + anti-MNS4	2 / 13	
- anti-MNS3	1 / 13	
- anti-MNS3 + anti-FY5		

Table 2: History of antibody detection and antibodies detected on screening at inclusion (* the Abs of low clinical significance are: autoantibodies and/or natural Abs and/or antibodies against LP antigens not related to Afro-Caribbean populations (RH8 (C^w), KEL3 (Kp^a), LU1 (Lu^a) and YT2 (Yt^b)) and/or Abs of unspecified specificity; †one or more associated Abs of low clinical significance may also be detected).

Table 3:

	Age (y)	Sex	History of Ab detection	Ab screening at inclusion	Anti-LP Ab detected during this study	Number of pRBC units received*
P1	21	M	auto-Ab	negative	anti-MNS6 + anti-RH23†	318
P2	44	M	anti-MNS3, -FY5, -YT2, anti-LE1, auto-Ab	anti-RH3 + anti-MNS1+ US Ab	anti-MNS6	5
P3	33	F	anti-RH3 + US Ab	anti-RH2 + auto-Ab	anti-RH30	78
P4	34	F	anti-RH2, -JK2, auto-Ab	negative	anti-RH23†	30
P5	34	M	none	negative	anti-RH23	165
P6	54	M	auto-Ab	negative	anti-RH30†	181
P7	31	F	anti-RH20, -KEL6	negative	anti-RH23	4
P8	13	F	none	negative	anti-RH23†	120
P9	39	F	anti-RH2, -RH3, -RH8, -RH10, -RH20, -RH23, -RH32, -FY3, -LE1, -KN, auto-Ab	anti-KN	none	29
P10	31	F	anti-RH2, -RH3, -MNS3, -RH20, auto-Ab	anti-RH2, -RH3, -RH5	none	23
P11	15	F	anti-RH2, -RH20, auto-Ab	US Ab	none	205 (90 before the detection of anti-RH20)

Table 3: Characteristics of patients presenting Afro-Caribbean-specific antibodies, detected during this study (P1 to P8), or recorded in the patient's medical history (P7 and P9 to P11). (* all pRBCs were received before the detection of Abs against Afro-Caribbean LP antigens, except in P11, † only detectable with papain-treated test RBCs; US: unspecified specificity, LP: low prevalence)

Table 4:

Antigen	Antigen prevalence in populations of African descent			Frequency of intra-ethnic transfusions ¹⁹	Antigen exposure risk †
	1000	211 French	Reported		
	French donors*	SCD patients*	by other authors		
RH10 (V)	NT	37%	30%	11.5%	2.68%
RH20 (VS)	45.8%	44.1%	26 to 40%		2.94%
RH23 (D ^W)	NT	NT	1% - 3.6%		0.11 – 0.41%
RH30 (Go ^a)	NT	NT	2%		0.23%
KEL6 (Js ^a)	17.2%	20.9%	20%		1.56%
MNS6 (He)	NT	NT	3 - 7%		0.33 – 0.78%

Table 4: Antigen exposure risk for RH10, RH20, RH23, RH30, KEL6, MNS6 antigens, expressed per pRBC unit received (* prevalence determined by molecular analysis in this study, † as detailed in Methods, NT: not tested)

Table 5:

Antigen	Calculated exposure rate, per pRBC unit received (%)	Alloimmunizations in our cohort: <i>n</i> (5%)
FY1 (Fy ^a)	53.6 %	5 (2.4%)
JK2 (Jk ^b)	40.1 %	10 (4.7%)
MNS3 (S)	38.6 %	9 (4.3%)
RH10 (V)	2.68 %	1 (0.5%)
RH20 (VS)	2.94 %	4 (1.9%)
RH23 (D ^w)	0.11 - 0.41 %	6 (2.8%)
RH30 (Go ^a)	0.23 %	2 (0.9%)
KEL6 (Js ^a)	1.56 %	1 (0.5%)
MNS6 (He)	0.33 - 0.78 %	2 (0.9%)

Table 5: Antigen immunogenicity, illustrated by the comparison between exposure and alloimmunization rates for FY1, JK2 and MNS3 antigens and Afro-Caribbean LP Ag.

FIGURE:

Figure 1: Study work flow

SUPPLEMENTAL DATA

Figure S1: RH10 and RH20 phenotype, deduced from the molecular analysis of positions c.733, c.1006 and c.712 of the *RHCE* gene. The most frequent alleles are presented.